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PRO-INFLAMMATORY CYTOKINE TRANSCRIPT LEVELS ARE ASSOCIATED WITH CD3δ EXPRESSION IN THE OSTEOARTHRITIC SYNOVIAL MEMBRANE

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Purpose: The aim of this study was to determine if inflammation within the synovial membrane of patients with OA was associated with increased expression of cytokines implicated in the osteoarthritic disease process. Many patients with osteoarthritis (OA) develop inflammatory infiltrates containing mostly macrophages and T-lymphocytes within the synovial membrane. However, whether the infiltrating cells play an active role in propagation of cartilage damage is unclear. Although these cells are capable of producing pro-inflammatory cytokines such as IL-1β and TNF-α, many other cells within the joint, including synovial fibroblasts and articular chondrocytes, are also potential sources of these mediators as well.
Methods: Synovial membrane (SM) specimens from 37 patients undergoing total hip or knee arthroplasty (n=31) or knee arthroscopy (n=6) for the treatment of OA were obtained at the time of surgery. RNA was extracted from SM specimens after tissue homogenization using a commercially available purification kit (Gentra Systems, Inc. Versagene™ RNA Tissue kit). cDNA was synthesized from $\leq$ 1 μg of total RNA. Relative expression levels (normalized to GAPDH) of CD3δ (as a measure of T-cell numbers), IL-1β, TNF-α, IL-15, IL-6, and TGF-β, were determined using quantitative real-time PCR analysis. The Spearman correlation test was utilized to determine if transcript levels of cytokines varied with the level of CD3δ expression.
Results: Detectable cytokine transcript levels were quantified in all specimens, using lps-stimulated mononuclear cells as the standard reference. As expected, expression of all cytokines analyzed was lower in the OA synovial membrane specimens than in the lps-stimulated mononuclear cells. Relative expression levels of CD3δ correlated most significantly with IL-1β transcript levels ($r=0.677$, $p<0.0001$). In addition, levels of TNF-α ($r=0.487$, $p=0.003$), IL-15 ($r=0.524$, $p=0.001$), and to a slightly lesser extent TGF-β ($r=0.414$, $p=0.015$) were associated with CD3δ transcript levels. IL-15 transcript levels were also highly associated with TNF-α expression levels ($r=0.739$, $p<0.0001$).
Conclusions: mRNA expression levels of the pro-inflammatory cytokines IL-1β, TNF-α, and IL-15 were significantly associated with levels of CD3δ transcripts within the SM of patients with both knee and hip OA. IL-1β and TNF-α are known to promote degradative enzyme synthesis and inhibit Collagen II synthesis in chondrocytes, and IL-15 has been linked to MMP-9 expression. The relationship between expression of these cytokines and CD3δ indicates that synovial infiltrating T-cells can potentially contribute to disease pathogenesis in OA by promoting synthesis of these pro-inflammatory cytokines.

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REGULATION OF NITRIC OXIDE AND BMP-2, TGF-β, SOX-9 EXPRESSION BY HYDROSTATIC PRESSURE IN SYNOVIAL FIBROBLASTS FROM RAT TEMPOROMANDIBULAR JOINT

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Purpose: Recent clinical evidence has suggested that inflammatory mediators and bone morphogenetic proteins may play a role in the pathogenesis arthritic diseases of the temporomandibular joint, such as rheumatoid arthritis and osteoarthritis. This project examines the effect of hydrostatic pressure on nitric oxide production and the expression of BMP-2, TGF-β and SOX-9 in synovial fibroblastic cells from rat temporomandibular joint.
Methods: Synovial fibroblastic cells derived from the double bilaminar zone of rat temporomandibular joint were grown to confluency in DMEM medium supplemented with 10% fetal calf serum. The monolayer of fibroblasts was subjected to different hydrostatic pressure (0kPa, 30kPa, 60kPa, and 90kPa) in a computer-controlled pressure chamber for 12 h. Nitrite concentration in synovial fibroblastic cells culture medium was measured spectrophotometrically using Griess reaction. Levels of TGF-β, BMP-2, and SOX-9 were examined by immunocytochemistry and Western blot approach.
Results: Exposure of synovial fibroblasts to a mechanical pressure for 12 h resulted in the release of accumulation of NO in the culture medium and increased NO release by 1.3-, 2.2- and 3.5-fold with 30kPa, 60kPa and 90kPa. Compared with the unpressurized control, level of BMP-2 expression increased by 18%, 29%, and 36% under different pressure conditions. The expression of TGF-β also gradually increased by 11%, 37% and 74%, accompanying with the raise of hydrostatic pressure, whilst expression of SOX-9, an important mediator of chondrogenesis, was upregulated by 27%, 37%, and 45%.
Conclusions: These data suggested that biomechanical stress-induced NO release and TGF-β may influence the differentiation of synovial tissue to chondrocyte in degenerative joint diseases and take part in the remodeling of bilaminar zone of temporomandibular joint after suffering biomechanical pressure.

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DIFFERENTIAL REGULATION OF CELL DEATH IN OSTEOARTHRITIC (OA) SYNOVIOCYTES BY TUMOR NECROSIS ALPHA (TNFα) AND INTERLEUKIN 1 β (IL-1β)

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Purpose: Osteoarthritis (OA) is a common cartilage and joint disease related to age which is characterized by a reduction in the number of chondrocytes, the loss of existing cartilage extracellular matrix, and the synovial inflammation. It is known that, in the last phases of OA, the synovial membrane plays an important role in the progression of the pathology. Tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) have been demonstrated to play a pivotal role in the development of OA disease. In fact, TNF-α and IL-1β differentially regulate the apoptotic pathway in human chondrocytes human.
Objective: This work is addressed to test whether TNF-α and IL-1β differently modulate the cell death in human synoviocytes.
Methods: Human OA synovium was obtained from 12 patients who were undergoing hip joint replacement. Ro 31-8220 (Ro, 10 μg/ml) were used to induce apoptosis in synoviocytes. Cell death were evaluated by using flow cytometry (propidium iodide) and nuclear morphology was evaluated with 4',6'-Dianidino-2-phenylindole dihidrochloride (DAPI) by fluorescence microscopy. As a control, cell death was induced in Jurkat cells with staurosporine (1μM). Caspase-7, -3 and bcl-2 were analyzed by Western-blot.
Results: The mitogen kinase phosphatase 1 inhibitor promoted synoviocytes cell death by flow cytometry, DNA fragmentation by DAPI, down-regulation of antiapoptotic protein bcl-2 and caspase-7 and -3 activation by western blot. We demonstrated that the increase of cell death induced by Ro was amplified by TNF-α but not by IL-1β (Ro: 4.88±0.06%; TNFα+Ro: 31,15±2,56%; IL-1β+Ro: 5.15±0,92%) at 24 hours. Nuclear morphological analysis of synoviocytes treated with TNFα+Ro resulted in a high number of cells condensed nuclei, not ob-